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NITRIFICATION IN ACID SOILS

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NITRIFICATION IN ACID SOILS.[1]

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It is quite generally believed now that nitrification may occur in acid soils, at least to some extent. The addition of lime, however, results in practically all cases in an increased activity of the nitrifying flora. This is especially true when soils are strongly acid in reaction and low in organic matter and when ammonium sulfate is used to measure nitrification.

Probably it is not merely because lime neutralizes acid soil conditions that it increases nitrification. There may be very important indirect effects of liming which are responsible for greater nitrifying action. Lime makes the soil conditions more favorable for the development of the nitrifying organisms and at the same time makes them less satisfactory for what may be called "acid-tolerant organisms" and an entirely different soil flora undoubtedly results. This new soil flora probably has a much less injurious effect on the nitrifying bacteria and may even stimulate their development. There are also many important physical and chemical effects of liming which indirectly affect nitrification. Undoubtedly the influence of lime on nitrification in general represents the combined effects of the material on the chemical, physical and biological soil conditions, all of which are very closely related.

HISTORICAL

The importance of nitrification, which is the making of nitrogenous material available to plants, has led to extended studies of the process, including its relation to the soil reaction. Early workers generally believed that acid soil conditions practically inhibited nitrification. In fact, it was believed that not many bacteria of any kind were active when soils became strongly acid. Even the ammonification occurring in acid soils was attributed to molds which are not so sensitive to acidity as are the bacteria.

Temple (18) found, however, that soils acid to the extent of a 3000 pound lime (CaO) requirement showed an appreciable nitrifying power. He showed also that organic nitrogenous ma-

1 Acknowledgements are extended to Dr. P. E. Brown for suggestions and assistance in the carrying out of this work and for reading the manuscript.

terials were nitrified much more extensively than a physiologically acid salt, such as ammonium sulfate. It may be suggested here that the explanation for the results leading to this latter conclusion may lie largely in the neutralizing effect of the ammonia which is produced in excess from the organic materials. In other words, the ammonia might be considered to take the place of calcium carbonate in satisfying the demands for a base.

Kelley (13) found that nitrates were not present in Hawaiian soils but calcium carbonate failed to induce nitrification. Evidently acidity was not the sole cause of the lack of the process in those soils.

Hall *et al.* (10) suggest that nitrification does not occur in acid soils except in local areas which are basic. They attribute the injurious effect of soil acidity chiefly to the inhibition of nitrification and of other essential bacteriological processes.

Boussingault and Breal (2) found that forest and meadow soils did not contain nitrates, presumably because of acidity developed in those soils.

Coville (4) concludes that the lack of the proper growth of plants on acid soils is due to nitrogen hunger, which is a result of suspension of the nitrifying process.

White (20) found that nitrification occurred in rather acid soils in Pennsylvania. For example, it took place when the soil showed a lime requirement of 8,373 pounds per acre according to the Veitch method. He suggests the possibility of the organisms becoming adapted to acid conditions. The application of ammonium sulfate to field soils in his experiments led very quickly to the production of acid conditions, the extent of acidity bearing a direct relation to the amount of fertilizer applied.

Fred (6) isolated the nitrifying organisms from Wisconsin soils which showed as high a lime requirement as 20,420 pounds per acre and he found that the cultures of these organisms were able to bring about nitrification when tested in solution. He showed also that organic nitrogenous compounds were nitrified much more rapidly in acid soils than was ammonium sulfate. Acid sands and peats showed a low nitrifying power even when limed, but acid loams permitted of a much more vigorous nitrification when treated with lime. Ammonium sulfate was nitrified more rapidly in the presence of lime, while casein was nitrified faster without it. Furthermore, more organisms were present where lime and organic materials were used and, therefore, greater nitrate assimilation took place under these conditions.

The above results indicate that acid soils not only have a nitrifying flora but that nitrification may occur to an appreciable extent. The work reported in the following pages was planned to throw more light on this problem.

EXPERIMENTAL

The soil used in the experiment was a Carrington loam, loose and friable in texture, and possessing an excellent tilth. It originally showed a lime requirement of nearly three tons of lime per acre, as tested by the modified Tacke method. The organic matter content was somewhat higher than that of average good soil. Previous cropping had shown that the field produced good grain crops but that legumes did not thrive. Just before the soil was sampled for this study, the field had produced a good crop of corn.

Pot studies were carried out in the greenhouse, using four gallon earthenware jars as containers. Lime in various amounts was added to the soils in duplicate pots. Lime was used at the rates of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15 and 20 tons per acre. Moisture conditions were maintained as nearly as possible at the optimum by frequent watering. Samplings were made at intervals of 2, 2, 4, 4, 4, and 7 weeks, covering a period of 23 weeks in all. Samples were taken from the pots by removing the surface soil, mixing the remaining portion, and sampling from the mixed soil. Tests were made for nitrifying power at each sampling. Duplicate 100-gram portions of soil from each of the pots were weighed out in tumblers, 100 mgs. of ammonium sulfate added to each tumbler and the moisture content adjusted to the optimum. The soils were then incubated for 39 days, when the amounts of nitrate present were determined. The lime requirements and the residual carbonates were determined in the soils at each sampling. The modified Tacke method was used for the determination of the lime requirement, and the residual carbonates were determined by decomposing with dilute acid and titrating the carbon dioxide liberated. A second series of pots, 20 in all, one gallon in capacity, were filled with the same soil and the same lime treatments made, except that the 6, 8, 10 and 15 ton quantities were omitted. Alfalfa was grown in these pots to determine the effect of the various applications of lime on this crop. The plants were allowed to grow until they reached the blooming stage. The crop was then harvested from all the pots on the same dates and the weights secured.

NITRIFICATION

For the determination of the nitrates, the Davis (5) modification of the phenoldisulphonic acid method was used. A preliminary test in which a known amount of nitrate was added to the soil showed that the method gave accurate results when rather large amounts of nitrates were present. Nitrate added at the rate of 100 parts of nitrogen per million of soil was recovered completely, as shown in table I.

TABLE I—THE AMOUNT OF NITRATE RECOVERED FROM TWO SOILS BY THE DAVIS METHOD

Soil	Nitrogen in Orig. Soil	Nitrate added	Nitrogen recovered
	P.P.M.—Nitrogen	As $NaNO_3$ P.P.M.—Nitrogen	P.P.M—Nitrogen
No. 1	2.7	100	103.4 102.8
No. 1a	2.7		103.3 102.5
AVERAGE	2.7	100 + 2.7 = 102.7	103.0 error 0.3 percent
No. 2	3.1	100	105.9 105.9
No. 2a	3.3		106.7 105.9
AVERAGE	3.2	100 + 3.2 = 103.2	106.1 error 2.9 percent

The amounts of nitrates present in the soils at the various samplings are given in table II.

This table reveals the fact that while there is considerable variation in the results, heavy liming tended in general to reduce slightly the amount of nitrates present in the soils. This was probably due to a greater assimilation of nitrates by bacteria in the presence of lime. It is seen also that in nearly all cases the greatest amounts of nitrates were found at the last

TABLE II—NITRATES IN THE ORIGINAL SOILS AT THE VARIOUS SAMPLINGS

Expressed in parts of Nitrogen per million parts of soil

Treatment	Pots	1st	Av.	2nd	Av.	3rd	Av.	4th	Av.	5th	Av.	6th	Av.	Av. of all
Nothing	A	30.0		19.6		33.9		37.6		47.3		45.9		
	B	27.4	28.7	28.2	23.9	32.1	33.0	36.8	37.2	43.6	45.4	34.8	40.3	34.8
1 ton $CaCO_3$	A	17.4		22.5		30.9		34.6		41.0		28.6		
	B	33.3	25.3	27.8	25.1	29.6	30.2	35.7	35.2	33.0	37.0	44.1	36.4	31.5
2 tons $CaCO_3$	A	30.6		24.5		39.7		34.8		38.9		43.8		
	B	19.7	25.1	25.2	24.9	29.2	34.4	35.4	35.1	33.7	36.3	48.6	46.2	33.7
3 tons $CaCO_3$	A	13.3		19.5		31.1		34.2		29.1		48.8		
	B	16.9	15.1	21.0	20.3	28.8	29.9	34.6	34.4	34.5	31.8	30.5	39.6	28.5
4 tons $CaCO_3$	A	32.1		24.5		35.5		42.2		41.7		46.6		
	B	31.0	31.5	25.2	24.9	28.9	32.2	41.8	42.0	33.4	37.5	37.4	42.0	35.0
5 tons $CaCO_3$	A	25.1		21.2		43.6		40.9		45.8		45.7		
	B	23.7	24.4	25.2	23.2	36.1	39.8	40.6	40.8	40.7	43.7	50.7	48.2	36.7
6 tons $CaCO_3$	A	28.4		27.7		30.3		37.5		38.9		39.2		
	B	33.4	30.9	27.9	27.8	33.3	31.8	37.8	37.6	37.9	38.8	27.9	33.6	33.4
7 tons $CaCO_3$	A	34.8		21.8		33.4		37.4		41.0		34.6		
	B	33.5	34.1	24.5	23.2	28.1	30.7	35.1	36.8	28.1	34.5	41.6	38.1	32.9
8 tons $CaCO_3$	A	15.7		20.7		29.8		35.2		30.6		39.4		
	B	25.4	20.5	27.7	24.2	31.4	30.6	34.8	35.0	44.9	37.8	52.5	45.9	32.4
9 tons $CaCO_3$	A	27.6		24.5		29.2		30.1		29.1		41.3		
	B	27.4	27.5	27.9	26.2	29.6	29.4	39.4	34.7	41.1	35.1	43.1	42.2	32.5
10 tons $CaCO_3$	A	28.2		20.7		27.4		29.8		40.9		42.0		
	B	17.0	22.6	19.7	20.2	26.8	27.1	25.2	27.5	30.6	35.8	44.7	43.3	29.4
12 tons $CaCO_3$	A	23.8		23.3		32.8		36.6		38.5		46.0		
	B	19.1	21.5	18.2	21.2	24.9	28.8	36.3	36.5	39.8	39.1	50.7	48.4	32.6
15 tons $CaCO_3$	A	21.0		23.5		20.4		24.2		33.4		45.5		
	B	19.8	20.4	23.3	23.4	25.5	22.9	35.6	29.9	39.0	36.2	34.9	40.2	28.8
20 tons $CaCO_3$	A	13.5		21.8		25.4		32.4		27.0		41.6		
	B	27.2	20.4	25.4	23.6	28.9	27.1	34.4	33.4	31.8	29.4	44.8	43.2	29.5
													Average	32.5

sampling. The greenhouse conditions were evidently favorable for the nitrifying process. The addition of large quantities of lime apparently did not increase the nitrification of the organic matter in the soil. Apparently lime should not, therefore, cause any marked loss of organic matter from normal soils, provided it is applied as the carbonate. The increase in numbers of organisms and their assimilation of nitrates may in fact tend to reduce the losses by leaching.

Examining table III, it appears that there were greater irregularities in the nitrification of ammonium sulfate in the soil in tumblers than were found in the original soil in the pots. Here, however, lime increased nitrification considerably. Three and four tons of the carbonate doubled the amount of nitrates produced and there was some increase in the production of nitrate with the increasing amount of lime, even up to the 20 ton treatment. This would indicate that a non-acid soil, or possibly one containing considerable carbonates, might respond to lime. To what the stimulative effect of such large amounts of lime may be due cannot be definitely stated. It is possible that each lime particle may represent local points of nitrification. If this were true, the greater application would supply a larger number of nitrifying centers and, therefore, a somewhat greater aggregate nitrification. This is in accord with the results of other workers who have shown that increased fineness of division of the limestone used caused greater nitrification.

Evidently the neutralization of the original soil acids is not the only factor to consider, for a comparatively small quantity of lime is sufficient for that purpose.

With larger applications than six to seven tons of lime, increases in the amount of nitrate produced were much less marked, and with additions of nine and ten tons nearly as much nitrate was produced as when the application was doubled. There is, evidently, a point beyond which greater additions of lime have little or no effect on nitrification.

Taking the average of all samples shown in table II, the maximum amount of nitrates was found in the original soil with the four and five ton applications of lime. A rather consistent increase is also shown which each successive sampling in the soils under various treatments. This is doubtless due to the increasingly favorable conditions for nitrification. In the tumbler experiments, averaging the results with all treatments, there was an increase in nitrates at each of the first three successive samplings, followed by a drop at the next two and an increase again at the last. These fluctuations may be without significance, but it is possible that they are partially the result of the effect of the lime on the bacterial species relationships.

The averages given at the bottom and in the right column of the table represent a considerable number of determinations and should be of more significance than the individual figures.

TABLE III—NITRIFICATION OF $(NH_4)_2SO_4$ IN SAMPLES DRAWN AT INTERVALS OF 2-2-4-4-4 AND 7 WEEKS

		SAMPLINGS						
Treatment	Pots	1st	2nd	3rd	4th	5th	6th	General Aver.
Check	A	Lost	69.8	79.4	81.4	77.6	67.5	75.0
		64.8	68.2	79.6	92.0	95.5	63.6	
	B	67.6	59.2	81.6	72.4	75.0	91.0	
		44.6	71.7	81.1	93.2	75.9	89.9	
1 ton $CaCO_3$	A	88.2	67.7	80.2	93.0	78.8	108.6	86.5
		57.0	95.3	80.6	80.9	86.0	108.6	
	B	104.1	82.2	102.5	102.8	107.6	76.7	
		77.8	90.0	98.6	95.5	107.8	95.4	
2 tons $CaCO_3$	A	103.5	68.3	132.6	77.2	98.8	123.1	118.3
		89.4	124.9	101.8	154.3	113.8	99.5	
	B	125.6	117.7	120.3	101.1	123.0	141.9	
		174.3	120.7	122.0	155.6	123.1	127.1	
3 tons $CaCO_3$	A	146.0	147.2	147.8	104.2	125.7	143.9	143.7
		126.8	139.4	154.3	158.7	142.5	134.6	
	B	129.9	145.2	150.4	156.2	146.1	162.3	
		46.7	137.5	161.5	152.2	134.6	155.5	
4 tons $CaCO_3$	A	154.5	175.0	178.9	128.7	Lost	148.5	157.8
		152.1	175.3	165.7	158.6	172.0	156.8	
	B	129.3	156.0	174.2	166.2	103.5	164.4	
		161.6	163.3	155.5	171.1	158.3	173.2	
5 tons $CaCO_3$	A	162.8	160.6	172.9	143.1	114.6	194.9	165.5
		161.5	200.0	156.3	172.3	143.4	168.9	
	B	164.2	164.4	177.9	170.6	123.4	181.1	
		162.9	184.4	170.3	168.7	169.7	182.4	
6 tons $CaCO_3$	A	178.8	161.4	194.4	175.5	160.2	165.4	171.4
		158.2	192.4	164.9	Lost	160.2	197.3	
	B	161.1	156.9	174.2	156.6	152.1	191.8	
		159.2	184.0	162.9	182.6	158.3	204.3	
7 tons $CaCO_3$	A	172.4	190.1	192.7	170.4	182.7	179.3	180.9
		157.8	199.9	172.0	189.7	179.4	195.5	
	B	173.7	173.0	199.9	177.0	178.9	181.9	
		162.5	184.7	177.5	172.8	178.9	199.0	
8 tons $CaCO_3$	A	199.9	176.1	181.6	168.1	184.9	186.4	182.1
		191.5	198.6	196.0	166.2	185.4	208.9	
	B	181.8	157.9	199.8	170.1	155.1	195.5	
		181.8	178.5	190.0	161.3	180.0	174.5	
9 tons $CaCO_3$	A	179.6	177.1	187.3	168.8	183.0	187.1	181.6
		179.6	192.6	191.0	168.5	195.9	179.3	
	B	177.2	161.2	192.9	165.5	174.2	179.0	
		188.2	192.2	203.2	184.3	172.1	180.0	
10 tons $CaCO_3$	A	202.4	169.2	196.6	150.2	184.2	187.0	188.0
		181.9	202.3	192.7	195.0	180.9	182.8	
	B	211.9	182.5	187.2	167.8	178.6	184.4	
		187.4	202.4	198.8	199.6	183.5	193.3	
12 tons $CaCO_3$	A	206.8	182.9	181.4	186.1	174.3	182.9	189.0
		184.8	199.4	197.1	178.7	174.3	190.9	
	B	212.5	177.5	194.7	185.8	189.5	192.0	
		180.9	205.0	211.4	174.3	183.7	189.4	
15 tons $CaCO_3$	A	209.6	197.8	215.4	188.0	182.5	187.6	191.6
		196.5	197.7	210.1	171.6	185.1	191.3	
	B	197.4	188.9	198.3	180.4	183.8	194.8	
		193.0	196.0	204.5	161.5	187.2	178.6	
20 tons $CaCO_3$	A	217.1	212.5	224.4	190.7	187.0	197.5	193.1
		196.5	199.4	217.8	185.4	186.0	182.1	
	B	199.0	197.6	222.3	172.3	Lost	182.8	
		195.9	197.6	209.5	172.4	145.5	191.1	
Average		156.1	160.2	167.3	155.0	151.8	163.5	155.3

WHY DO ACID SOILS NITRIFY?

Recent theories of the relation of the process of nitrification to acid soil conditions have been considerably modified. The nature of soil acidity is better understood and it is now believed that the concentration of the hydrogen-ion is the fundamental cause of acidity. The presence of acid salts, relatively insoluble acids and amphoteric substances, may cause the amount of base taken up by the soil to be high, when there is really only a small concentration of active acids. Potential acidity and the hydrogen-ion concentration of the soil are certainly widely different at times, and this difference may well occur in most cases. There is undoubtedly a difference in the effect of active acidity and potential acidity on nitrification.

Furthermore, nitrates are nearly always found to some extent even in rather acid soils. It is possible that the nitrifying organisms may be more resistant than has been believed formerly. Probably also other bases than carbonates function locally and permit nitrification to occur to some extent.

Molds have been found to be rather efficient ammonifiers and these organisms endure extremely acid conditions. Practically no soil, therefore, should be too acid for ammonification to occur. When ammonia is produced, acidity is neutralized and at each point of production of ammonia, nitrification may begin. Any ordinarily acid soil may therefore be expected to contain the nitrifying organisms and to nitrify to a limited extent at least. The reasons given also explain why organic materials such as casein may nitrify more readily in acid soils. That is, the ammonia produced is removed so rapidly by the soil acids that a toxic accumulation cannot occur as soon as may be the case in the limed soils. The neutralization of the acidity by the ammonia gives also a more suitable reaction for the functioning of the nitrate organisms. There are doubtless other factors to consider, however, for some nitrogenous organic materials have been found to be nitrified more readily in the presence of lime. Ammonium sulfate always nitrifies more readily when lime is supplied, but here there is the double effect of the sulfuric acid liberated and of the nitric acid produced.

It is possible, too, that organisms may endure a greater acidity in soils than in culture media. The colloidal materials of the soil, such as the proteins, acting as buffers, serve to protect the organisms and they also supply food for the bacteria.

The common method of reporting soil acidity is in terms of lime requirement, rather than in hydrogen-ion concentration, which represents the active acidity. The hydrogen electrode is the most reliable method yet developed for determining the hydrogen-ion in soils, but it has not been used extensively. In a few instances, acidity has been reported in terms of normality,

regardless of hydrogen-ion concentration. Hall, Miller, and Gimingham (10) report easily soluble acids extractable by washing, equivalent to 1/60th to 1/140th normal. When this is compared with n/10 hydrochloric acid, which is about 90 per cent ionized and gives a hydrogen-ion concentration of nearly $9x10^{-2}$ gm. ions per liter, it seems very probable that the soil acid extracted, which was equivalent to 1/6 to 1/14 as great a normality, gave a very much lower hydrogen-ion concentration than that theoretically possible, probably lower than that of acetic acid which is about 1/70th as great as for hydrochloric acid of the same concentration. On the basis of the ionization of acetic acid, the 1/60th normal extraction should have represented a hydrogen-ion concentration in the soils of approximately $2.4x10^{-4}$ gm. ions of hydrogen. This would represent a rather acid soil, while on the basis of the ionization of hydrochloric acid the concentration should be about $1.5x10^{-2}$, which is more acid than soils probably become. Such an extracted acidity would perhaps be the result of acids, some fairly strong, others very weak. While the weak acids could be titrated, they might be completely suppressed from ionization by the stronger acids.

The hydrogen-ion concentration found by Sharp and Hoagland (16,12) varies from $2x10^{-4}$ in very acid soils to $2x10^{-10}$ in alkaline soils. Plummer (15), in studying the film of hygroscopic water in the soil, where the soil organisms are generally active, found that the hydrogen-ion concentration there was somewhat greater than in the soil suspension in the case of acid soils, while in basic soils the hydroxyl-ion concentration was greater. Considering all available data along this line, it would seem that the soil acidity usually reported is much larger than that which would be represented by an equivalent in hydrogen-ion concentration, and the acidity of the soil film is not often determined.

The acidity found in very acid soils, as suggested above, is about 1/450 that of n/10 hydrochloric acid, which is considered a dilute solution. But many soil organisms are doubtless killed or retarded by even a much weaker acidity than this. Reports given by several workers (1, 4, 6, 7) have shown many organisms to be sensitive to hydrogen-ion concentrations of 10^{-5} and10^{-6}. Gruzit (9) found that the general flora of the soil solution from a sand culture was subjected to a germicidal toxicity by an acid concentration of n/1200 when sulfuric and hydrochloric acids were used. A concentration of n/2164 acids caused the destruction and increase of bacteria to balance each other. This latter acidity is roughly equivalent to a $4x10^{-4}$ hydrogen-ion concentration, which is very close to the acidity of the more acid soils. The same worker found also that the toxic limits for corn seedlings, when the above acids were used, resulted in a 43 per-

cent reduction in the number of soil bacteria present. This data would indicate that acidity may cause a direct injury both upon plants and upon organisms, and that the extent of the injury depends in part at least upon the hydrogen-ion concentration. A discussion of the toxic hydrogen-ion concentrations in relation to various organisms may be found in a publication by Fred (6).

There can be little question but that certain very important organisms are quite sensitive to acidity. Thus it is reported by Gainey (8) that azotobacter were not found in soils with a hydrogen-ion concentration of 10^{-6}. This is only a mildly acid soil and therefore it would seem that the solution of the nitrogen problem, by increasing azofication, is dependent very largely upon the reaction of the soil. The nitrifying organisms are also quite sensitive to acidity, but it is always possible for such organisms to be active in local areas of slight acidity. It may be possible also that chemical actions occurring within the soil films are modified by osmotic and surface energy in such a way that organisms are not as readily affected as under less natural conditions. There is data to show that densities are modified appreciably (as much as 0.5 percent) when substances are sufficiently finely divided and that increasing the fineness of division greatly increases solubility. Since all these factors and many others are operative in soils, it is not possible to determine the exact environment to which soil organisms are subjected. The organisms themselves have effects that cannot be measured. The carbon dioxide which they produce increases the surface energy of the soil films, while the organic substances which they help put into solution lower the surface energy. The death of organisms is brought about by chemical actions upon the protoplasm of the organism. The chemical energy within the soil film water, expressing itself in certain reactions, is much modified by the surface energy of the film and hence there is a close but indefinite relationship between the toxicity of the soil acids and their various effects.

A few facts stand out prominently in the above discussion. First, a soil may be theoretically too acid for nitrification and yet it is very seldom that the organisms and some nitrates are not present. The smallest quantities of nitrates are usually found in water-logged soils where aeration would be a limiting factor for the nitrifying organisms. Second, several tests have shown an appreciable nitrification in soils of very high acidity. As high as 10,000 and even 20,000 pounds lime requirement have been reported when nitrates were present. Soils also which contained scarcely any nitrate, presumably on account of acidity, were found to nitrify in solution, proving the presence of the organisms.

ARTIFICIAL ACIDITY

The following study of nitrification in soils made acid by additions of sulfuric acid was carried out in tumblers. Two soils were used, both slightly acid at the start, and made strongly acid by adding varying amounts of acid. One soil was of the same type, tho not from the same field, as the soil used in the pot studies. The other was quite sandy and low in organic matter. It is probable that sulfuric acid should give a fairly high hydrogen-ion concentration in the soil and that its toxicity would be due to this ion rather than to the sulfate ion. A higher hydrogen-ion concentration should be produced with the sandy soil, because of lack of organic matter and consequent lack of buffering. Therefore, the acid should be more toxic on the sandy soil. Below are the results showing the lime requirement by the modified Tacke method (17), since no method was available for the determination of hydrogen-ion concentration.

Table IV shows that the results were as predicted. The sandy soil was more severely affected by a similar addition of sulfuric acid. Both soils show a wonderful response to lime, but considering the lower nitrifying power, the sandy soil really shows the greater effect. The water extract of all these acid-treated soils showed a high degree of acidity by the litmus test and also by the color given to methyl orange, which is proof that there was no reaction by which the acid was removed from active functioning by the soil. It is quite remarkable that the loam showed considerable nitrates present with as high a lime requirement as twelve tons, while the other soil showed little with six and a half tons. These figures indicate that the statement that a soil has a lime requirement of 5 to 10 tons has little significance. Nearly twice as much nitrate is present with a 12 ton requirement on

TABLE IV—SHOWING ACIDITY AND NITRATES AFTER FIVE WEEKS' INCUBATION

Loam Soil	Acidity Tons	P.P.M. Nitrate Nitrogen
Soil alone	4.20	97.1 99.7
Limed	0.90	201.6 225.9
H_2SO_4 added	5.70	78.7 72.7
H_2SO_4 added	7.10	55.1 52.1
H_2SO_4 added	8.55	46.9 49.3
H_2SO_4 added	10.90	37.9 31.2
H_2SO_4 added	12.00	39.1 34.7
H_2SO_4 added	20.40	None

Sandy Soil	Acidity Tons	P.P.M. Nitrate Nitrogen
Soil alone	2.85	66.5 59.5
Limed	0.20	208.3 227.3
H_2SO_4 added	4.50	62.5 24.8
H_2SO_4 added	5.10	18.4 19.5
H_2SO_4 added	6.50	8.9 6.2
H_2SO_4 added	9.95	Trace
H_2SO_4 added	11.05	Trace
H_2SO_4 added	19.90	None

the loam as with a 5 ton requirement on the sandy soil. On both soils, however, the decrease in nitrates follows quite regularly the increase in acidity.

A second study was made to determine what effect lime and acid might have upon a soil which was only slightly acid. The test was made with ammonium sulfate and the tumblers were incubated 42 days. The results are shown in the table V.

The test shows the same general result. Lime increased nitrification very markedly and the larger application brought about the greater increase. Again nitrification occurred in the presence of the added sulfuric acid, but only to a slight extent.

In table VI appear the results of nitrification tests on different soils. The object of this experiment was to determine whether an increase in nitrification would result from liming a soil already containing carbonates. On one soil casein was used to test for nitrification, while on the other two ammonium sulfate was employed. One acid soil was tested by the use of casein, one lime treatment being made. For the nitrification of ammonium sulfate, soils rich in carbonates were used, but lime was applied also.

The above data indicate that it is possible to add sufficient acid to check ammonification, but nitrification ceases where a light application of phosphoric acid is made. In this test it may be observed that lime has checked both ammonification and nitrification, where casein is used. On the soils containing the large amounts of natural carbonates, the application of lime increased nitrification, but only to a small extent. If a soil were only neutral or contained just a small excess of carbonate, it might be expected that nitrification would be stimulated by liming. Such a soil was not available, however, for use in this work. Since the nitrifying organisms are very sensitive to acidity, some basic material must be present before nitrification can occur. With only a small amount of base the supply is soon exhausted and nitrification is sooner checked than when a larger amount is present. The more lime and the more finely it is divided, therefore, the greater is the nitrification in the soil, up to a certain limit. This limit would doubtless vary for different soils, but it is probably several tons per acre in most cases.

TABLE V—THE EFFECT OF LIME AND ACIDS UPON NITRIFICATION

Treatment	Total Acidity Tons	P.P.M. Nitrates as Nitrogen
Soil alone	2.3	30.3
Soil + 10 tons $CaCO_3$	....	111.1
Soil + 20 tons $CaCO_3$	....	153.8
Soil + H_2SO_4	9.2	Trace
Soil + H_2SO_4	16.2	Trace
Soil + H_2SO_4	60.2	None

TABLE VI—NITRIFICATION IN VARIOUSLY TREATED SOILS, INCUBATED 43 DAYS

Treatment	Tons Acidity	P.P.M. Ammonia N.	P.P.M. Nitrate N.
Soil + Casein	1	772.8	176.4
Soil + Casein + Lime	10	649.6	62.5
Soil + Casein + H_3PO_4	4.8	817.6	Trace
Soil + Casein + H_3PO_4	24.7	84.0	Trace
Soil + Casein + H_2SO_4	20.6	117.0	Trace
Soil with 50 tons natural carbonates + $(NH_4)_2SO_4$	...	....	120.0
Soil with 50 tons natural carbonates + 10 tons $CaCO_3$ + $(NH_4)_2SO_4$	...	....	134.4
Soil with 50 tons natural carbonates + 20 tons $CaCO_3$ + $(NH_4)_2SO_4$	...	....	120.0
Soil with 15 tons natural carbonates, alone + $(NH_4)_2SO_4$	...	....	147.3
Soil with 15 tons natural carbonates + 10 tons $CaCO_3$	...	....	176.4
Soil with 15 tons natural carbonates + 20 tons $CaCO_3$	...	....	176.4

SOIL ALKALINITY

The question whether a soil may be too alkaline for bacterial life is of considerable interest. Quick-lime is sometimes employed for partial sterilization and evidently restricts bacterial growth. Calcium carbonate, however, probably acts very differently from quick-lime. The concentration of soluble salts which is commonly termed alkali is not under consideration here. Such conditions would undoubtedly retard or completely inhibit normal bacterial activity. Hoagland has reported that in nutrient solutions, an equal divergence of the hydroxyl-ion concentration from the neutral point caused greater toxicity than did an abnormally high hydrogen-ion concentration, when barley seedlings were grown. The response of organisms to variations in reaction would not be identical, but might be very similar. A hydroxyl-ion concentration of 2.5×10^{-5} was distinctly toxic, while a hydrogen-ion concentration of 0.7×10^{-5} was favorable to growth. Only when the hydrogen-ion concentration was 3×10^{-4} did serious toxicity occur.

In recent work by Hoagland and Christie (11), a hydroxyl-ion concentration as low as $10^{-2.76}$ is reported where calcium oxide (0.5 per cent) was added to the soil. This is very alkaline and tho possible to produce, does not occur, it may safely be said under field conditions. The same workers found that calcium carbonate added to the soil gave an alkinity of 10^{-6}, which is perhaps close to toxicity, but this concentration did not endure for long and would probably cause no injury. The quicklime treatment, however, did cause sterilization and no nitrification occurred. It may be mentioned also that Gruzit (9) found an alkalinity of 10^{-4} somewhat injurious to organisms in sand cultures. The

effect of lime in general, however, seems to depend very much upon the soil. After a few days, any alkalinity produced by lime has usually become much less marked and has continued to become smaller as time elapsed. Plummer (15) reports that calcium carbonate may increase the alkalinity, but not to an injurious extent. In this work heavy applications have been made, but the addition of 20 tons to soil already containing 50 tons of carbonate did not depress nitrification. The results from the alfalfa grown on the pots likewise would not warrant any general statement of toxicity.

Considering all the data, it may safely be said that practically never would a toxic alkalinity be produced by liming, especially with calcium carbonate.

ACIDITY RESULTS

The acidity changes are given in table VII, as indicated by the modified Tacke (17) method. Determinations were not made at all samplings, since the amount of lime requirement indicated had become nearly constant in some cases and the data was therefore not essential.

Examining table VII, it appears that the lime requirement of the soil was about three tons, the average of the five tests being 2.75 tons. The addition of lime up to 4 tons per acre diminished the acidity. From 4 tons on, a practically constant acidity is shown. The variations which occur at different samplings and with different determinations are not large, considering the many opportunities for errors in sampling the soil. The table shows also that there was very little difference in the lime requirement at the third and fifth samplings, so that no information was lost by failing to run tests at the fourth sampling. The reaction

TABLE VII—LIME REQUIREMENT IN TONS OF CALCIUM CARBONATE PER ACRE, AT THE VARIOUS SAMPLINGS [1]

Treatment	1	2	3	5	6	Aver.
0	2.80	2.80	2.70	2.65	2.70	2.75
1 ton $CaCO_3$	2.40	2.45	2.15	2.05	2.50	2.31
2 tons $CaCO_3$	1.15	1.05	1.05	1.35	1.30	1.38
3 tons $CaCO_3$	1.00	0.90	0.70	1.05	0.70	0.87
4 tons $CaCO_3$	0.75	0.80	0.75	0.87	0.50	0.73
5 tons $CaCO_3$	0.85	0.70	...	0.75	0.45	0.55
6 tons $CaCO_3$	0.80	0.65	...	0.80	0.40	0.53
7 tons $CaCO_3$	0.70	0.70	0.75	0.65	0.55	0.67
8 tons $CaCO_3$	0.75	0.75	0.65	0.65	0.45	0.53
9 tons $CaCO_3$	0.90	0.70	0.60	0.60	0.55	0.67
10 tons $CaCO_3$	0.95	0.75	...	0.60	...	0.57
12 tons $CaCO_3$	0.95	0.70	...	...	0.40	0.68
15 tons $CaCO_3$	0.85	0.75	0.75	...	0.65	0.75
20 tons $CaCO_3$	0.80	0.75	0.80	...	0.50	0.71
Average from 4 tons down	0.83	0.73	0.71	0.70	0.50	0.64

[1] The lime requirement was not determined at the fourth sampling.

between the lime and the soil acids has evidently taken place rather rapidly. It may be presumed that the more reactive acids were neutralized at the end of two weeks. From that time on there was a gradual using up of base. The entire test covered 23 weeks. During the last seven weeks, 400 pounds of carbonate were apparently used up.

The question may arise why the method showed a lime requirement in all cases. The answer is that soils do not react under field conditions to permit complete neutralization. This is due to the fact that soils are not thoroly enough mixed to secure intimate contact and that slowly soluble acids develop locally either by hydrolytic processes or otherwise. Decay naturally produces some acidity, at least temporarily. Then, too, there must be an entire absence of lime at local points until the soil and lime have been for a long time in contact. The results show that after a long contact only half a ton of acidity remained, regardless of whether little more than enough lime was supplied to neutralize the soil acids, or whether a large excess was supplied. Of course, when the soil is brought into intimate contact with carbonate in the shaking machine, more complete neutralization would be expected, and tests show that it occurs.

As proof of the above suggestions, tests were made with the Truog (19) qualitative test. It was found that in all tests, the soils receiving a two ton application of lime gave a slight indication of acidity, while from three tons on the reaction was basic.

This shows again that three tons of lime, the amount indicated by the modified Tacke method, was sufficient to neutralize the active acidity of the soil. The qualitative test evidently shows when sufficient lime has been added to satisfy the more active acidity, regardless of whether or not complete neutralization has yet occurred.

RESIDUAL CARBONATES

In table VIII are given the results of the determinations of residual carbonates in the variously treated soils in the greenhouse. The carbonates were determined by decomposing with phosphoric acid (1-15) and collecting the carbon dioxide evolved.

A study of the table reveals the fact that there was a gradual using up of carbonates up to the last sampling, but that by far the greatest demand came at once. The averages are taken for all treatments above three tons, since that was approximately the lime requirement of the soil. There has been, therefore, a very rapid neutralization of the active acids. The method indicates a lime requirement of 2.75 tons. After deducting the residual carbonates from the original application, it is found that 2.8 tons, almost identically the average lime requirement of the untreated soil, have been used in two weeks by the soil. As the qualitative test indicates that this amount has neutralized the

TABLE VIII—RESIDUAL CARBONATES IN TONS OF $CaCO_3$

Treatment	1st Sampling	2nd Sampling	3rd Sampling	5th Sampling	6th Sampling	Amt. $CaCO_3$ used up 1st Sampling	Amt. $CaCO_3$ used up 6th Sampling
1 ton	0.15	0.05	0.00	0.00	0.00	0.85	1.00
2 tons	Lost	0.20	0.30	0.30	0.00	Lost	2.00
3 tons	0.95	0.70	0.55	0.25	0.10	2.05	2.90
4 tons	1.45	2.25	1.25	0.70	0.15	2.55	3.85
5 tons	2.75	2.00	2.10	1.60	0.70	2.25	4.30
6 tons	3.15	2.30	2.80	2.85	1.40	2.85	4.60
7 tons	4.20	3.60	3.25	3.85	2.65	2.80	4.35
8 tons	5.60	4.55	4.20	3.90	3.30	2.40	4.70
9 tons	6.50	5.80	5.90	4.55	4.00	2.50	5.00
10 tons	6.80	6.45	5.70	5.80	5.55	3.20	4.45
12 tons	9.50	7.95	7.50	7.10	6.60	2.50	5.40
15 tons	11.90	10.25	11.50	10.60	11.10	3.10	3.90
20 tons	16.45	16.10	15.10	14.80	14.20	3.55	5.80
Aver.	6.80	6.10	5.90	5.60	5.00	2.80	4.60

soil acids, and as enough determinations have been made to remove most of the possibilities of error, it seems evident that the active acids have been correctly estimated and that a three ton application of lime would be an abundance for this soil.

The next question that arises is, what became of the excess carbonate which was used up as the experiment proceeded. The data shows that during the second two weeks an additional 1,400 pounds were used; during the four weeks following, 400 pounds more; during the succeeding eight weeks an additional 600 pounds and finally, during the last seven weeks, 1200 pounds were used up by the soil, until there is a total of a little more than 4½ tons used. Part of the high figures are doubtless due to errors in determining such very large amounts of carbonates. There is no doubt, however, but that the soil has used a considerable excess of carbonate over the indicated needs. The reason for this probably is that very insoluble and little ionized acids have gradually reacted. Furthermore, the nitrogen changes which have occurred have made increased, tho small, demands for base, up to the last sampling. And some organic acids must have developed while the process of nitrification has been occurring and these would use some of the base temporarily. Still another cause for the disappearance of base is its fixation by the soil mineral complexes and thus, to a limited extent, its change to silicates (14) which were not reactive with the phosphoric acid used in determining the carbonates. The data indicates that soils may undoubtedly use carbonate in considerable excess of the demands for neutralization of active acidity.

BACTERIA IN THE SOILS

In this study only very limited bacteriological examinations were made, and not a great significance attends the results. The

TABLE IX—THE NUMBER OF ORGANISMS IN MILLIONS PER GRAM ON GELATIN PLATES

Treatment	SAMPLINGS			
	1st	2nd	3rd	4th
Check	1.12	2.86	4.76	4.76
1 ton $CaCO_3$	2.02			
2 tons $CaCO_3$	2.53			
3 tons $CaCO_3$	2.93			
5 tons $CaCO_3$	2.48	3.84	...	5.06
6 tons $CaCO_3$	3.57			
7 tons $CaCO_3$	2.68			
9 tons $CaCO_3$	2.62			
10 tons $CaCO_3$	2.52			
12 tons $CaCO_3$	3.00			
20 tons $CaCO_3$	3.14	3.94	5.80	4.56

gelatin medium used probably does not permit of the development of any of the nitrifiers. Nevertheless, many of the organisms which are plated cause ammonification and this process must precede nitrification, so that undoubtedly there is an indirect relationship. Large numbers of organisms mean greater competition and vice versa, and this is a factor in any activity.

In this study only a few platings were made, mainly at the first sampling and then at various later intervals, primarily to determine what effect the lime had upon numbers. The results are given in table IX.

In general the effect of the lime was to increase the number of organisms. The effect was more marked at the first sampling than later. The number of organisms increased proportionately more without the lime than with it, and has more than doubled in many cases. The increase is due doubtless to favorable conditions of moisture and temperature. Evidently the acidity of such a degree as occurs in this soil is not very toxic to common soil organisms. This is probably more especially true of soils such as this one, which is relatively high in organic matter and in good physical condition.

POT STUDIES WITH ALFALFA

The pot studies with alfalfa were rather inconclusive. The pots were treated in the same way as for the bacteriological studies and alfalfa was seeded thickly. By successive thinning the number of plants per pot was finally reduced to five. This selection largely eliminated differences due to individuality of plants, and should permit each treatment to exhibit its maximum production.

All plants were thrifty in appearance and growth, and it was not possible to detect any difference until toward the end of the experiment, when some of the treatments seemed to forge ahead. The plants were harvested soon after blooming started and both green and dry weight determined. A summary is given in table X.

TABLE X—GREEN AND DRY WEIGHTS OF ALFALFA

Green Weight	Treatment—Tons $CaCO_3$									
	0	1	2	3	4	5	7	9	12	20
	gms	gms	gms	gms	gms	gms	gms	gms	gms	gms
Pot 1	14.0	19.5	21.0	24.0	16.0	20.0	14.0	19.0	18.0	19.0
Pot 2	18.0	22.0	24.0	32.5	13.0	19.0	13.0	20.5	16.0	12.5
Dry Weight										
Pot 1	4.0	5.5	5.8	6.6	4.3	5.5	3.7	5.3	5.0	5.2
Pot 2	5.2	6.2	6.8	8.2	4.9	5.0	3.3	5.5	4.6	4.0

The table shows that in a general way the dry weights follow the same tendency as the green weights, but the effects are rather more marked. The maximum weight is produced with the three ton treatment, which is the lime requirement of the soil. The weight produced seems to increase gradually up to this point, after which there is a drop and no treatment in excess of this amount has produced much greater growth than the untreated soil. These results would indicate that too much lime might be just as injurious as not enough, but the data is too limited for definite conclusions.

The pots with the three ton treatment also bloomed first. In fact many of the others did not bloom for several days after. The pots were examined for nodules, which were found in considerable abundance, so that differences were not due to lack of inoculation. A second crop was allowed to mature and produce seed, but was not harvested. The same difference in rate of growth and time of blooming was noted as before. A considerable setting of seed occurred on the unlimed as well as on the limed pots and, in general, the second crop behaved in the same way as the first.

CONCLUSION

The results of this study give further proof of the capacity of acid soils for nitrification. Even when most of the soil contains a toxic hydrogen-ion concentration, there may be local areas of low intensity where nitrification can occur. It is usually true that soils reported highly acid contain a relatively low concentration of active acidity. Highly soluble acids which would be very active leach too readily to accumulate in soils, except as salts. Slowly soluble acids and amphoteric substances may cause a soil to show a high lime requirement and yet not produce a highly toxic acidity. An illustration of this is found when 4 to 5 tons of sulfuric acid added to the soil nearly stopped nitrification, while naturally acid soil of a higher lime requirement nitrified very readily.

The results of this work indicate that tho very large amounts of lime may give greater nitrification, only that which is necessary to neutralize the most active acids is essential for adequate nitrification and maximum crop production. Too rapid a nitrification is a wasteful process, because neither plants nor or-

ganisms can assimilate the nitrates as fast as produced. Fred and Graul (6) found in their work that only half the application of lime indicated by the Truog barium hydroxide method was necessary. The desired end is, of course, to stimulate nitrification only sufficiently to meet the needs for maximum crop production.

SUMMARY

1. Nitrification has been found to occur in the presence of a rather high lime requirement.

2. A scarcely measurable effect was produced in the nitrification of the original soil nitrogen, by the application of calcium carbonate. Lime did cause a marked increase, however, in the nitrification of ammonium sulfate.

3. The soil acids were never completely neutralized, even with the very heavy applications of lime, apparently because of the slow solubility of a part of the acidity present.

4. The amount of carbonate taken up by the soil, calculated upon the basis of residual carbonates, agreed well with the indicated lime requirement. After several weeks, however, more lime had been taken up than was equivalent to the requirement according to the modified Tacke method. This was because the method, permitting reaction to occur for only a short time, did not carry the equilibrium as far toward neutrality as nature did in a much longer time. But even after the longest period of time, an acidity seemed to have developed which was sufficiently reactive to be measured by the method. This apparent inconsistency means only that there is probably always a slowly reactive acidity in a soil, due to acid silicates, organic substances or other amphoteric colloids.

5. The growth of alfalfa was at a maximum when an amount of lime approximately equivalent to the indicated requirement was added. Growth was nearly as good without any lime as with the heavier applications.

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